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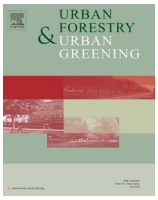

Long-term storage affects resource availability and occurrence of bacterial taxa linked to pollutant degradation and human health in landscaping materials

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ABSTRACT

Man-made landscaping materials form uppermost soil layers in urban green parks and lawns. To optimize effects of landscaping materials on biodiversity, plant growth and human health, it is necessary to understand microbial community dynamics and physicochemical characteristics of the landscaping materials during storage. In the current three-year study, the consequences of long-term storage on biotic and abiotic characteristics of eight commercial landscaping materials were evaluated. We hypothesized that long-term storage results in changes in microbial utilization of various energy sources and the diversity and relative abundance of bacteria with pathogenic or immunomodulatory characteristics. Three-year storage led to remarkable changes in bacterial community composition. Diversity and richness of taxa associated with immune modulation, particularly phylum Proteobacteria and class Gammaproteobacteria, decreased over time. Bacteroidetes decreased while Actinobacteria increased in relative abundance. Functional orthologs associated with biosynthesis of antibiotics and degradation of complex carbon sources increased during storage. Relative abundance of genera containing potential pathogens were mostly constant or decreased with time. Major changes can be explained by tightening competition over lessening resources. Bacterial communities in landscaping materials adjust to absent inflow of carbon and nutrients during storage. The increased signalling of functional orthologs related to degradation of complex carbon sources hints that bacteria dependent on labile carbon and readily available nutrients were outcompeted. This suggests storage reduces plant seedling growth. Long-term storage seems to decrease immunomodulatory potential of landscaping materials, but storage did not enrich pathogens or functional orthologs associated with pathogenicity. We recommend short storage and shelf life of organic landscaping materials.

1. Introduction

Landscaping materials contain a mixture of microbes, such as bacteria, that cooperate and compete with other microbes and plants. The microbial composition of the environment influences human health, as its microbiological content is mirrored in the human microbiome (Von Hertzen et al., 2011; Nurminen et al., 2018; Grönroos et al., 2019) and the microbial communities in homes (Parajuli et al., 2018; Hui et al., 2019b; Kirjavainen et al., 2019). Microbiomes of the skin and gut influence the regulation of human immune defense (Nurminen et al., 2018), and microbiome composition seems to be related to immune-mediated diseases, such as atopy (Hanski et al., 2012), and has also been shown to affect mental health (Valles-Colomer et al., 2019). Urban biodiversity is weakening in developed societies, leading to poor microbial exposure of city dwellers, which has been stated as the main cause for increasing autoimmune diseases and allergies in welfare states (Rook, 2009; Von Hertzen et al., 2011; Hui et al., 2019b; Kirjavainen et al., 2019).

Urban biodiversity can be increased by urban gardening (Orsini et al., 2013), greening public spaces (Hui et al., 2019a; Puhakka et al., 2019), and utilizing diverse plant communities and landscaping materials containing high microbial diversity (Parajuli et al., 2018, 2020).

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Certain bacterial groups have been linked to the development and regulation of the immune system, and the commensal metagenome has been associated with endocrine disruption (Von Hertzen and Haahtela, 2006; Roslund et al., 2019). The diversity of Proteobacteria on skin has been observed to be low among city dwellers, and people with atopy have low gammaproteobacterial diversity on their skin (Hanski et al., 2012). In the same study, the abundance of *Acinetobacter* (a gammaproteobacterial genus) on skin correlated positively with anti-inflammatory cell signals in healthy non-atopic study subjects (Hanski et al., 2012). In the gut, bacteria that produce butyrate (butanoate) are beneficial for health (Geirnaert et al., 2017) and mental well-being (Valles-Colomer et al., 2019).

Gardening and landscaping materials are typically stored outdoors or packed in plastic sacks for retail sellers. To optimize plant growth and utilization of microbially diverse substrates for adjusting microbial exposure among city dwellers, shifts in the abundance of functional orthologs (i.e. functions of a bacterial community estimated from genomic data) and microbial community composition during storage of landscaping materials should be taken into consideration for determination of "microbial shelf-life" of landscaping materials. Microbial shelf-life in this context means changes in microbial community dynamics linked to resource availability, the diversity and relative abundance of bacterial taxa associated positively with human health, and the low occurrence of bacterial functions and taxa indicating pathogenicity. In boreal climate, the degradation of complex carbon sources often takes years and phyla, such as Actinobacteria, are characteristic of the later stages of succession due to their ability to degrade complex organic molecules (Cutler et al., 2014; Herzog et al., 2019). Genera belonging to the phylum Actinobacteria, such as *Mycobacterium,* are known to utilize complex carbon sources and to include pathogenic species (Pagnout et al., 2007; Herzog et al., 2019). Indeed, incidents where people have contracted *Mycobacterium* infections from commercial soil products have been reported (De Groote et al., 2006). Importantly, in Herzog et al. (2019), root litter mass was degraded by approximately 40 % in two years. As bacterial communities in landscaping materials strongly reflect nutrient availability and as environmental bacterial communities have a distinctive, well-recognized potential in immunomodulation (Rook et al., 2004; Parajuli et al., 2017; Flandroy et al., 2018), we focused on bacterial community dynamics and prediction of functional orthologs using next-generation sequencing and Kyoto Encyclopedia of Genes and Genomes (KEGG). To further understand potential differences in bacterial community compositions between landscaping materials, we analyzed organic matter (OM), elements, water content, pH, bioavailable nutrients, and carbon and nitrogen content.

Since root litter has been observed to require more than two years to degrade (Herzog et al., 2019), and our study included composted plant-based materials containing root litter, we chose to investigate the long-term effects of storage in a three-year experiment. As far as we are aware, this is the first study to explore the effect of long-term storage (three years) on commercially produced, organic landscaping materials; previous studies have focused on effects of short-term storage on soil (e.g. Lauber et al., 2010; Rubin et al., 2013) from a scientific sample storage viewpoint. Our main hypothesis was that long-term storage intensifies competition, reduces bacterial diversity, and alters relative abundances of many taxa. Additionally, we hypothesized that species richness decreases, the relative abundance of health-associated (beneficial and pathogenic) bacteria declines, and functional orthologs indicating degradation of complex carbon sources and those of antibiotic synthesis become more abundant.

2. Materials and methods

2.1. Experimental design and sampling

Eight soil and plant-based materials (landscaping materials) were acquired in March-April of 2015 from commercial producers. The landscaping materials consisted of composted animal and plant waste, dried moss, and forestry side streams, as in Grönroos et al. (2019). The landscaping materials were termed MixComp (composted mixture of plant and fungus-based materials, poultry dung, and woody debris), PlantComp (composted plant and garden waste), LowNutrSoil (nutrient-poor mineral soil enriched only with peat), AOMsoil1 and AOMsoil2 (fertilized with animal waste-based OM), moss (dried moss), and SLOMsoil1 and SLOMsoil2 (fertilized with sludge-based OM). Landscaping materials were collected directly into 10 l containers from large piles. Five shovelfuls of each material were collected from random spots. Alternatively, the shovelfuls were first put into passively aerated polyethylene sacks that were transferred into 10 l containers (Appendix B) within one day. The container lids were perforated with two air holes to allow passive oxygen flow, as in Sinkkonen et al. (2013a). Thin pieces of cotton wool were placed in the holes to prevent fast drying. The containers were stored in a cold room (5−7 °C) from April 2015 to May-August 2018.

Before storage, each of the eight materials was sampled for bacterial DNA extraction, dry weight estimation, OM estimation, pH analysis, and soil characterization, as in Roslund et al. (2018). Briefly, the samples for DNA extraction were drawn from a depth of 2−12 cm and 2 cm away from the container edges. Within these limits, approximately 2 g of material was taken from five random spots and immediately pooled together and stored at -80 °C before extraction. For dry weight estimation, approximately 10 g of material was dried at 105 °C for 24 h or more if needed, as in Sinkkonen et al. (2013b). OM estimation was done according to the SFS 3008 (1990) standard. The pH was measured according to the SFS-ISO 10390 (2005) standard. Bioavailable nutrients (NH_4^+, NO_3-, and PO_4^{3-}) were determined as described in Sinkkonen et al. (2013b). Elements (Cd, Al, Co, Cr, Cu, Mn, Ni, Fe, Zn, P, As, Pb) were determined as described in Roslund et al. (2018). Carbon and nitrogen content was measured with the LECO C/N/S-200 analyzer (Leco Corporation, Saint Joseph, MI, USA). The materials were sampled again in the same way after storage for DNA extraction, dry weight estimation, pH analysis, and bioavailable nutrients.

2.2. Sample preparation and sequencing

The samples were prepared for MiSeq Sequencing, as in Roslund et al. (2018). In short, the bacterial DNA was extracted with a PowerSoil® DNA Isolation Kit (MoBio Laboratories, Inc., Carlsbad, CA, USA) according to the kit protocol with three technical replicates per sample. The amount of DNA (ng/mL) was quantified by Quant-iTTM PicoGreen® dsDNA reagent kit (Thermo Fisher Scientific, Waltham, MA, USA), and based on the results, the DNA samples were adjusted to 0.4 ng/ml prior to polymerase chain reaction (PCR) during which variable region V4 within the 16S ribosomal RNA (rRNA) gene was amplified. Forward primer was 505 F (5′-GTGCCAGCMGCCGCGGTAA-3`) and reverse primer 806R (5`-GGACTACHVGGGTWTCTAAT-3`) (Caporaso et al., 2012). A successful PCR process was assured for the PCR products with a positive control (Cupriavidus necator JMP134, DSM 4058) and with agarose gel (1.5 %) electrophoresis, after which the primers were cleaned from the PCR products with Agencourt AMPure XP solution (Beckman Coulter Inc., Brea, CA, USA). The cleaned PCR products were tagged for sequencing in secondary PCR by including 12 bp unique Multiplexing Identifier tags (MID-806R) into the reverse primer. The tagged PCR products were cleaned again with Agencourt AMPure XP. The samples were sequenced with Illumina MiSeq 16S rRNA gene metabarcoding using 2 × 300 bp version 3 sequencing kit according to the manufacturer's protocol at Kansas State University (samples before storage) and at the Institute for Molecular Medicine Finland (FIMM, samples after storage). The 2015 samples before storage were sequenced and submitted to the Sequence Read Archive, but not analyzed by Grönroos et al. (2019). The 2018 samples after storage were published under the same BioProject code (PRJNA393827) as the 2015 samples. For the 2018 samples, the primers were the same as in Hui et al. (2019a),

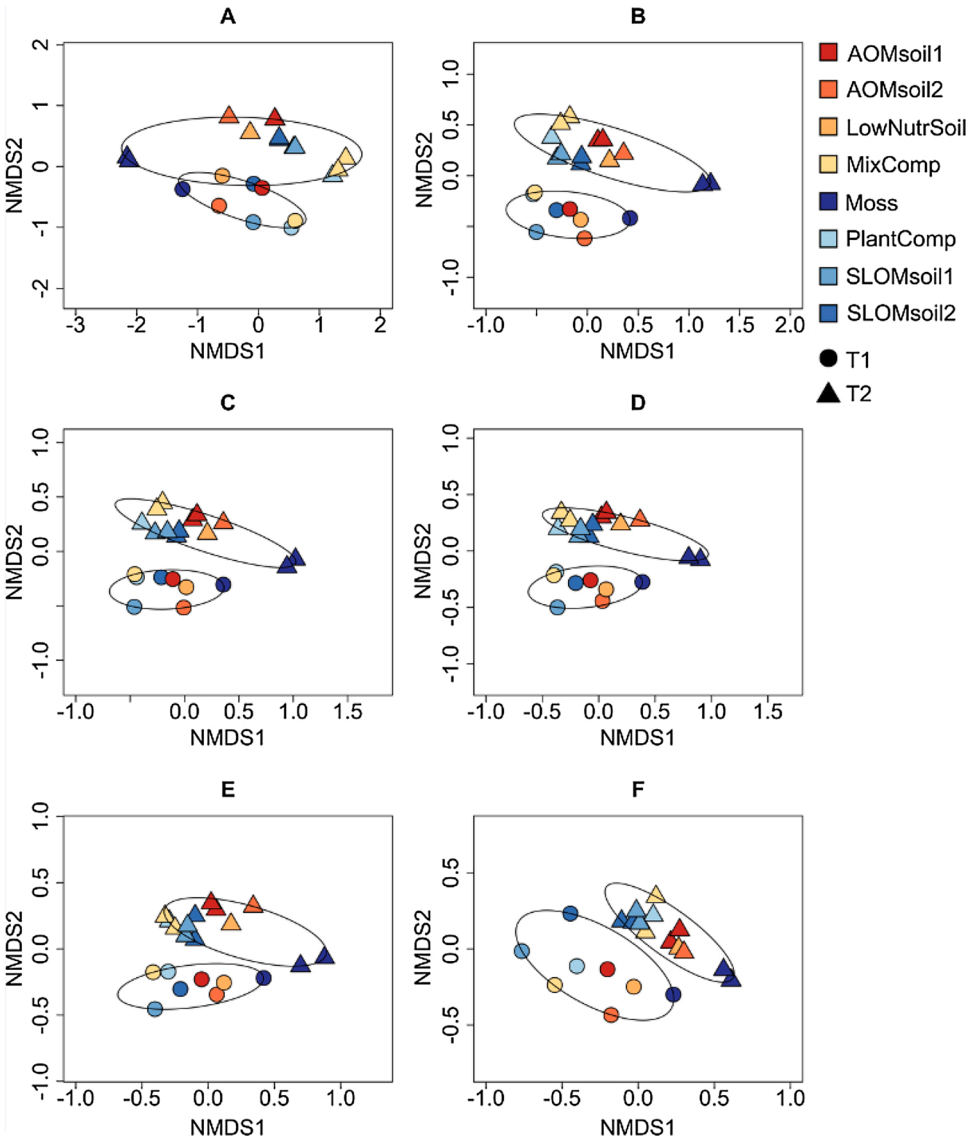

Fig. 1. NMDS ordinations were visualized for all taxonomic levels: A) OTU ($P = 0.001$, stress = 0.096), B) genus ($P = 0.001$, stress = 0.103), C) family ($P = 0.001$, stress = 0.093), D) order ($P = 0.001$, stress = 0.101), E) class ($P = 0.001$, stress = 0.108), F) phylum ($P = 0.001$, stress= 0.110). Colors indicate soil materials and shapes indicate timepoints T1-T2.

thus, the only difference between sequencing batches was that the 2018 samples were amplified with Illumina overhang primers. The primers were chosen according to the preferences of the two sequencers. A negative control for DNA extraction was prepared from sterile water and sequenced in the same batch with the samples. For PCR, negative (no sample) controls were also prepared and sequenced in the same batch.

2.3. Bioinformatics

For the most part, data were processed according to Schloss et al. (2011) and Kozich et al. (2013), as in Roslund et al. (2018). Briefly, the sequences were analyzed using Mothur (version 1.42.2.) with SILVA (version 123, Pruesse et al., 2007) as an alignment reference. The sequences were preclustered to avoid sequencing errors (Huse et al., 2010). Chimeras were searched by UCHIME (Edgar et al., 2011) and deleted. Remaining sequences were classified with Bayesian classifier (Wang et al., 2007) with the RDP training set version 16 (Cole et al., 2009) with 80 % bootstrap threshold. Non-bacterial sequences were removed. A pairwise distance matrix was calculated for unique sequences and OTUs clustered at 97 % sequence similarity. OTUs with 10 sequences or less were removed. Six abundant OTUs detected in negative controls and considered contaminants were removed. The Good's coverage index (average ± SD: 0.92 ± 0.04) as well as alpha diversity indices (Chao1, Shannon diversity, Simpson evenness, Inversed Simpson and observed taxon richness (Sobs)) were calculated using summary. single command by setting the subsampling value to 4796 according to the smallest sequence count found in the samples. Finally, the samples were subsampled to the 4796 sequence count and OTU data were drawn as a shared file for bacterial community analyses. The method used to identify the bacteria in this study produced taxonomic data to genus level, with a few OTUs identifiable at the species level. In parallel with taxonomic analyses, we predicted bacterial functions in the communities by defining functional orthologs on the basis of metabolic genes in the metagenome. A profile of putative bacterial functions was generated as in Hui et al. (2019a) from the 16S rRNA OTU data classified against Greengenes Database (DeSantis et al., 2006) with PICRUSt (Langille et al., 2013). KEGG (Kyoto Encyclopedia of Genes and Genomes, release 91.0) database was used to categorize functional orthologs indicating infectious bacterial disease, competition (biosynthesis or resistance to antibiotics, degradation of complex carbon sources), and health benefit

(butanoate metabolism) (Kanehisa and Goto, 2000). Anthroponotic diseases, such as tuberculosis, pertussis, and *Helicobacter pylori* infection, were excluded because we were not studying the survival but rather the advancement of genera containing opportunistic pathogens. As the goal of KEGG analysis was to understand major metagenomic shifts, orthologs weaker than 10 000 signals at both timepoints (T1, T2) were excluded from the analysis.

2.4. Statistics

R environment for statistical computing (version 3.6.1, R Core Team, 2019) was used for all statistical analyses. Major phyla, classes of Proteobacteria, abiotic factors (pH, water content, bioavailable nutrients, proportions of C and N), functional KEGG orthologs, and diversity indices were compared between timepoints using *t*-test when data were normally distributed according to the Shapiro-Wilk test, and with Wilcoxon test when the distribution was not normal. Since the samples were not drawn from exactly the same containers, we did not use paired tests. Some of the soil types (SLOMsoil1-2, Moss, AOMsoil1, and MixComp) had two replicates for the second timepoint (T1, n = 8, T2, n = 13). The replicate samples were combined into an average value before performing the tests. The difference in bacterial community composition between timepoints was visualized and analyzed using the vegan package (Oksanen et al., 2019); non-metric multidimensional scaling (NMDS) was performed (function metaMDS) alongside permutational multivariate analysis of variance (PERMANOVA, function adonis) using Bray-Curtis dissimilarity. NMDS ordinations were formed according to Bray-Curtis distances with default metaMDS settings and visualized. To find possible correlations between factors and bacterial community composition for individual timepoints, abiotic factors were fitted onto NMDS ordinations of the 50 most abundant OTUs (function envfit, vegan package), as in Roslund et al. (2019). In the case of pH and water content, one value was defined for each soil material. When these factors were fitted onto the ordinations, the same pH and water content value were used for replicate samples at T2. Both adonis and envfit functions were run with the default settings of 999 permutations. When multiple comparisons were conducted (analyses regarding KEGG, genera potentially containing opportunistic pathogens, and fitting abiotic factors onto bacterial data), the *P*-values were corrected according to the number of comparisons with the false discovery rate (FDR) method and are referred to as *Q*-values. The diversity indices of phylum Proteobacteria and class Gammaproteobacteria were determined from the subsampled data with phyloseq package (function estimate_richness) (McMurdie and Holmes, 2013) and compared between timepoints with *t*-test or Wilcoxon test, as in other taxonomic comparisons. In the case of potentially pathogenic genera, OTU data were screened by genus using the list of emerging human pathogens by Taylor et al. (2001) as a reference, as in Hui et al. (2019a). Low abundance OTUs (less than 3 sequences) were not included. Since data concerning genera had an abundance of zeros, comparison of genera with 10 or more zeros between timepoints was tested by Fisher's exact test (13 genera), and the data were first converted to binary form (present/absent). Potential pathogens that increased during storage were searched from NCBI GenBank using BLASTN version 2.10.0+ (Zhang et al., 2000) online at BLAST website. The relative abundance of genera with less than 10 zeros was compared with Wilcoxon test or *t*-test depending on their distribution (14 genera). Regarding comparison of major phyla and classes of Proteobacteria, the relative abundances were visualized with Circos-0.67−7 (Krzywinski et al., 2009).

3. Results

3.1. Bacterial community composition and diversity

Three-year storage resulted in shifts in bacterial community composition according to PERMANOVA analyses (Fig. 1). Interestingly, changes in moss were distinguishably different from those in the other landscaping materials. The composted materials also seemed to be differing from other landscaping materials at the OTU and genus levels.

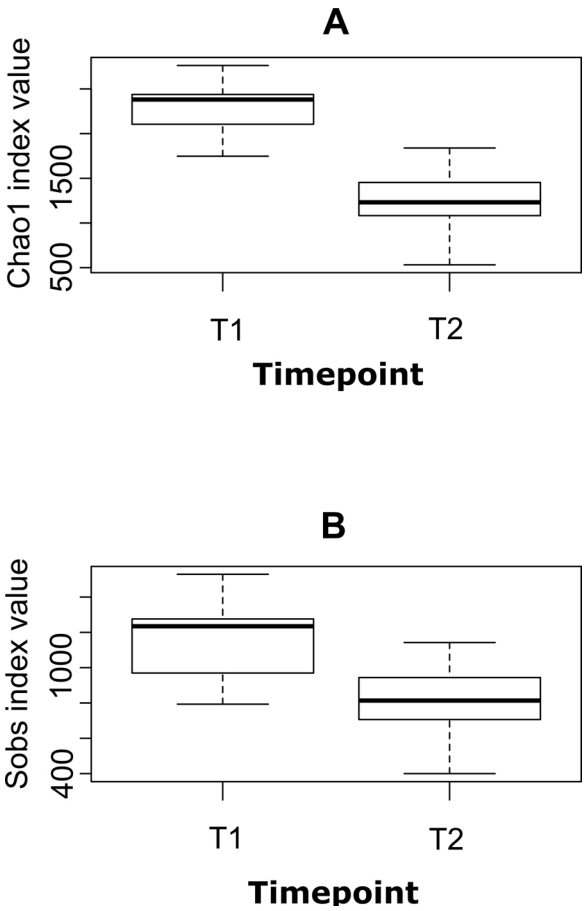

Fig. 2. Species richness indices changed by storage: A = Chao1 (t = 6, df = 13.6, P = <0.001), B = Sobs (t = 3, df = 13.9, P = 0.004).

The diversity indices Shannon, inverse Simpson, and Simpson evenness stayed unchanged over the three-year storage, while species richness indices (Fig. 2) Chao1 (t = 6, $P < 0.001$) and Sobs (t = 3, P = 0.01) were lower after storage. Thus, especially the number of OTUs with low abundance declined during storage. The diversity indices within phylum Proteobacteria and class Gammaproteobacteria followed the same decreasing trend: Chao1 (t = 6.19, $P < 0.001$) and Sobs (t = 3.5, P = 0.004) were lower after storage.

Relative abundances of major phyla (>1%) shifted during the three-year storage (Fig. 3). On average, Bacteroidetes was almost three times less abundant (t = 7.6, $P < 0.001$), while Actinobacteria (W= 0, $P <$ 0.001) and Planctomycetes (W = 1, P = 0.001) were almost five times more abundant and Acidobacteria almost twice as abundant as at the beginning of storage (W = 12, P = 0.038). The most abundant phylum Proteobacteria showed no change, but within Proteobacteria all classes except Deltaproteobacteria were affected by the storage (Fig. 4). On average, Alphaproteobacteria doubled during storage (t = -3.8, P = 0.004), Betaproteobacteria decreased by 39 percentage points (t = 2.7, P = 0.023), Gammaproteobacteria decreased by half (W = 59, P = 0.003), and low abundance class Epsilonproteobacteria was absent after storage (W = 64, P = <0.001).

3.2. Genera containing opportunistic pathogens

Most genera containing opportunistic pathogens either decreased or remained constant throughout the storage period. Using Taylor et al.

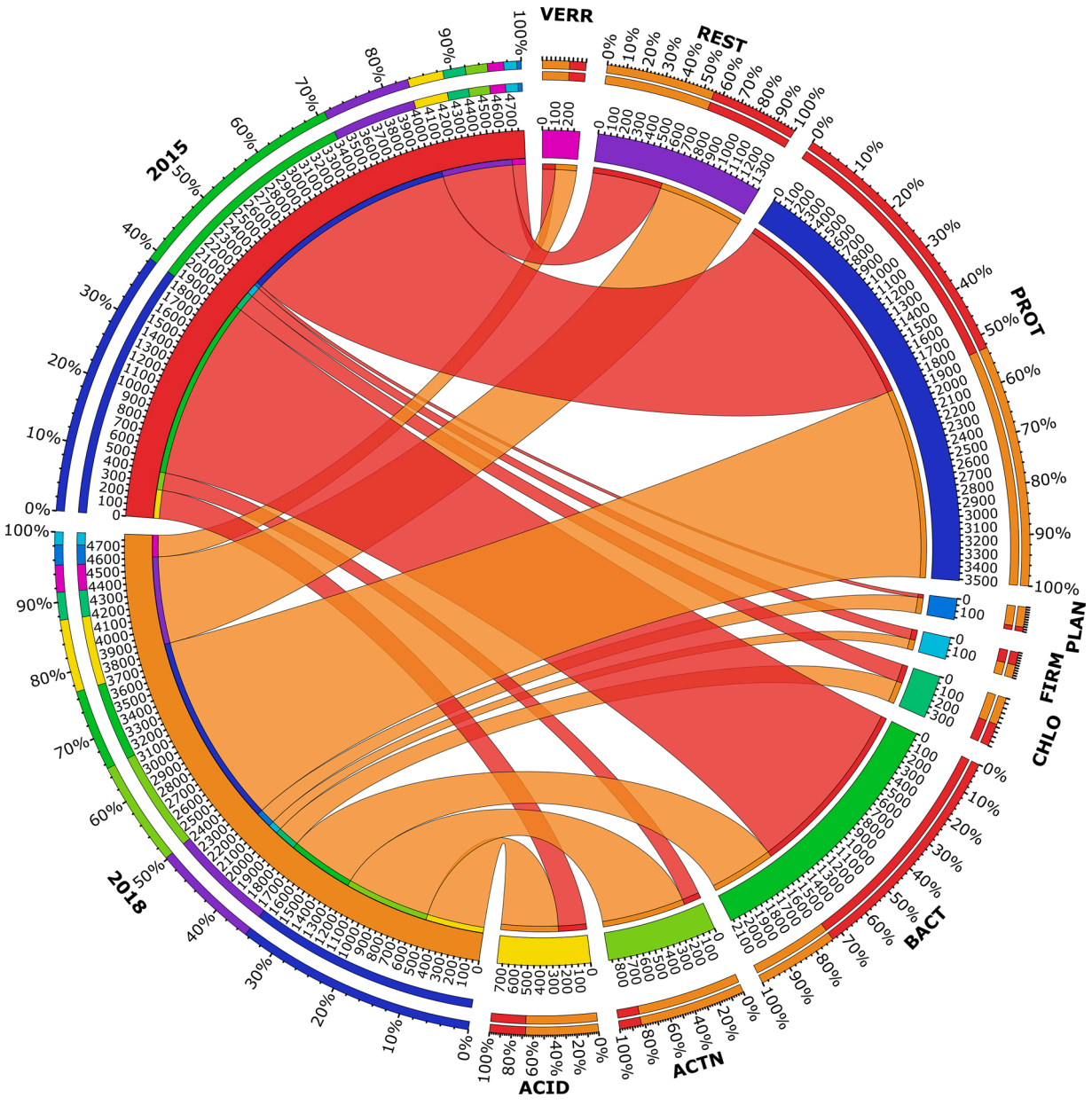

Fig. 3. Relative abundances were visualized for bacterial phyla between timepoints. ACID = Acidobacteria (**P*), ACTN = Actinobacteria (****P*), BACT = Bacteroidetes (****P*), CHLO = Chloroflexi, FIRM = Firmicutes, PLAN = Planctomycetes (****P*), PROT = Proteobacteria, VERR = Verrucomicrobia, and REST = combined abundance of minor phyla (relative abundance <1%).

(2001) as a reference (pathogen list) for potentially pathogenic genera, 36 matching genera were found within the data. The relative abundance of 5 genera changed during the storage (Table 1); *Pseudomonas*, *Chryseobacterium*, *Acinetobacter,* and *Comamonas* decreased and *Pseudonocardia* increased in abundance during storage. *Pseudonocardia* consisted of 4 OTUs and of its total observed abundance 98 % was observed after storage, representing 6% of all sequences found after the three-year storage. *Mycobacterium* was also elevated during storage, but the change was not significant after *P*-value adjustment; *Mycobacterium* had 6 OTUs and of its total observed abundance 95 % was observed after storage, representing 13 % of all sequences found after storage. Altogether, the relative abundance of genera potentially containing opportunistic pathogens was 7.71 % before and 3.23 % after storage.

According to the BLAST results, none of the OTUs classified as *Pseudonocardia* matched with *P. autotrophica* found on the pathogen list. Matches for *Mycobacterium* OTUs included species found on the pathogen list as well as species not found on the pathogen list and vague definitions such as uncultured bacteria and organisms. Two low-abundance *Mycobacterium* OTUs matched with species on the pathogen list; OTU377 (relative abundance 0.06 % of all sequences after storage) matched with *M. simiae*, *M. tuberculosis*, and *M. genavense*, and OTU613 (relative abundance 0.02 % of all sequences after storage) matched with *M. xenopi* and *M. shimoidei*. However, both OTUs also had BLAST matches outside the pathogen list. For OTU377, numerous mycobacterial matches were closer (identity 99.21 %, E-value 1e-124) or as close as *M. simiae*, *M. tuberculosis*, and *M. genavense* (identity 98.81 %, E-value 6e-123). Several mycobacterial matches were equally close to OTU613 as *M. xenopi* (identity 98.42 %, E-value 3e-121) or closer than *M. shimoidei* (Identity 97.23 %, E-value 3e-116).

The most abundant *Mycobacterium* in the samples was OTU110, representing 0.52 % of the after-storage (T2) sequences. The closest matches (Identity 100 %, E-value = 6e-128) were uncultured bacterium clones from environmental samples related to drinking water. The second closest matches (Identity 99.60 %, E-value = 3e-126) were several

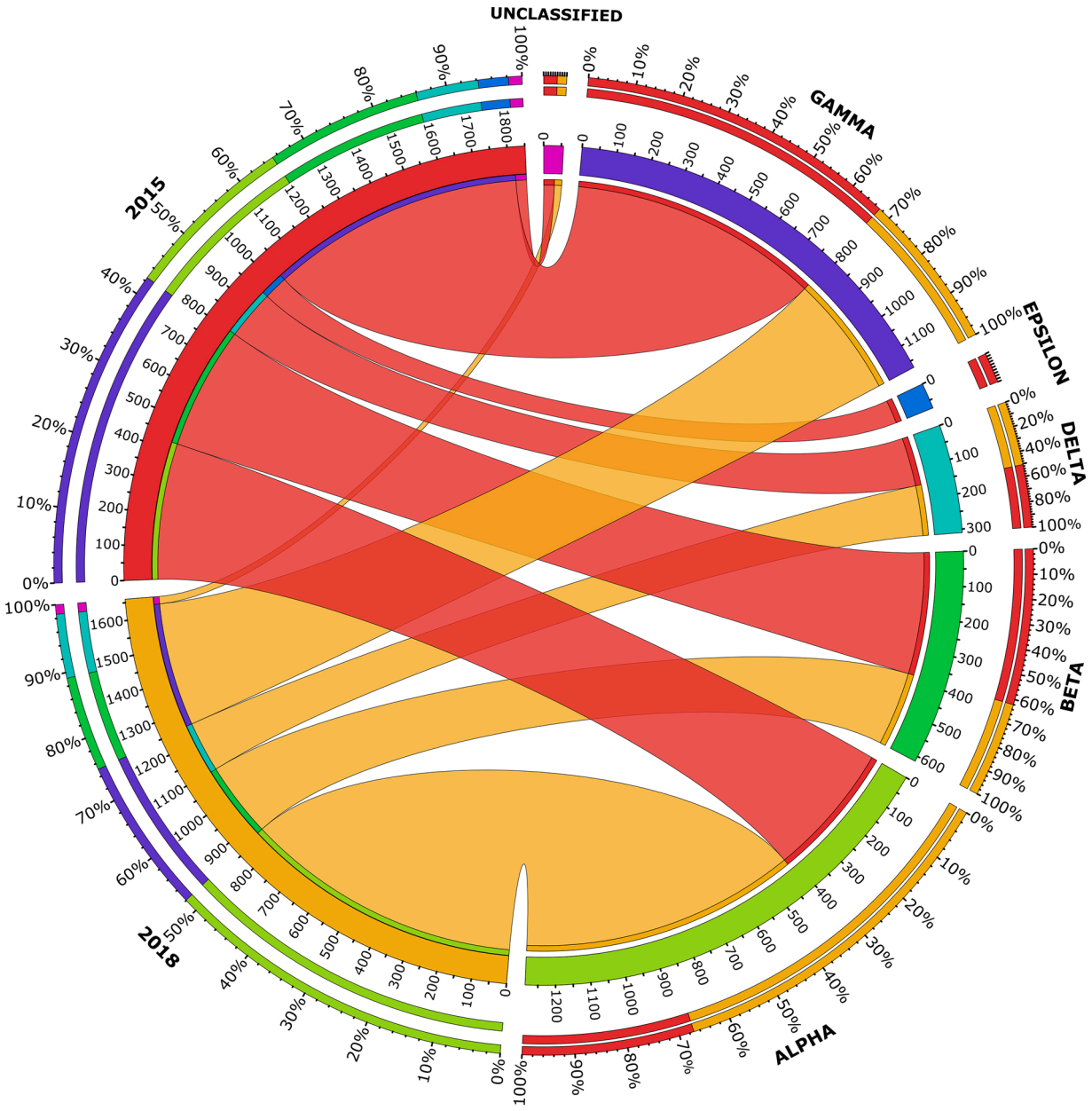

Fig. 4. Relative abundances of classes within phyla Proteobacteria were visualized between timepoints. Classes are marked as ALPHA = Alphaproteobacteria (**P*), BETA = Betaproteobacteria (**P*), GAMMA = Gammaproteobacteria (***P*), DELTA = Deltaproteobacteria, EPSILON = Epsilonproteobacteria (****P*), and UNCLASSIFIED = Unclassified Proteobacteria.

Table 1
Six genera potentially containing opportunistic pathogens changed in relative abundance during storage (Change: increase = + or decrease = -). The Wilcoxon test statistic (W) is followed by *Q*-value.

Genus	W	*Q*	Change
Chryseobacterium	64.00	0.005	–
Mycobacterium	10.00	0.054	+
Pseudomonas	63.50	0.005	–
Acinetobacter	64.00	0.005	–
Pseudonocardia	7.00	0.023	+
Comamonas	59.00	0.012	–

Mycolicibacter species and *Mycobacterium* sp. strains or *Mycobacterium* species not found on the pathogen list. The second most abundant *Mycobacterium* OTU200 represented 0.31 % of all sequences in the T2 samples. The closest matches (98.81−98.42%) were uncultured bacteria found in soil samples and *M. cookii* species not found on the pathogen list. More detailed match information for the BLAST results is presented in the Supplementary material.

3.3. Functional orthologs

Regarding functional KEGG orthologs, we were interested in orthologs related to nutrient recycling, infectious human diseases caused by bacteria, biosynthesis of or resistance to antibiotics, xenobiotic degradation, and butanoate metabolism. During the three-year storage an increase was observed in the signaling of several orthologs, especially in xenobiotic degradation and biosynthesis of antibiotics and butanoate metabolism (Table 2). Orthologs indicating infectious human diseases were absent from the data.

Table 2
KEGG orthologs indicating antibiotic biosynthesis (A), health benefit (H), and xenobiotic degradation (X) were compared between timepoints using *t*-test (t) or Wilcoxon test (W). *Q*-values of the tests show increase in the signaling of these orthologs during storage.

Ortholog	Test	*Q*	Indication
Biosynthesis of ansamycins	t	0.017	A
Butirosin and neomycin biosynthesis	W	0.005	A
Novobiocin biosynthesis	t	0.001	A
Streptomycin biosynthesis	t	0.008	A
Butanoate metabolism	t	<0.001	H
Aminobenzoate degradation	t	<0.001	X
Atrazine degradation	t	0.002	X
Benzoate degradation	t	<0.001	X
Bisphenol degradation	W	<0.001	X
Caprolactam degradation	t	<0.001	X
Chloroalkane and chloroalkene degradation	t	<0.001	X
Chlorocyclohexane and chlorobenzene degradation	t	<0.001	X
Dioxin degradation	W	<0.001	X
Ethylbenzene degradation	t	<0.001	X
Fluorobenzoate degradation	t	0.008	X
Naphthalene degradation	t	<0.001	X
Nitrotoluene degradation	t	<0.001	X
Polycyclic aromatic hydrocarbon degradation	t	<0.001	X
Styrene degradation	t	<0.001	X
Toluene degradation	t	0.006	X

3.4. Soil characteristics and bacterial community composition

Before storage, soil characteristics were determined for the soil materials. OM content varied between 2% and 99 % in the materials. Solely plant-based Moss was almost completely organic (99 %), whereas LowNutrSoil content was almost completely inorganic, as shown in Appendix A. The elements Cd and As were below the level of quantitation. The most abundant elements found in the materials were Fe, Al, and P (see Appendix A).

After three years, water content was reduced by 58 percentage points during storage (t = 2.4236, $P = 0.03$), but no change was observed in pH level (Fig. 5). Additionally, no change occurred in bioavailable nutrients or proportions of C and N in dry matter. Interestingly, the amount of NH_4^+ increased during storage in two materials (SLOMsoil2 and Moss) (Fig. 5). When these factors were fitted onto NMDS ordinations for timepoints separately, some factors correlated with differences in bacterial community compositions, especially after storage. The ordinations were plotted, including the factors alongside the samples and the 50 most abundant OTUs (Fig. 6).

Bacterial community composition before storage was correlated with pH ($r^2 = 0.8787$, Q-value = 0.02) and Cu ($r^2 = 0.8591$, Q-value = 0.03) (Fig. 6a). After three years (Fig. 6b), the effect of abiotic factors on bacterial community composition was pronounced. Water content ($r^2 = 0.76$, Q-value = 0.005), pH ($r^2 = 0.87$, Q-value = 0.004), NH_4^+ ($r^2 = 0.67$, Q-value = 0.015), and total C ($r^2 = 0.85$, Q-value = 0.004) and N ($r^2 = 0.76$, Q-value = 0.012) correlated with bacterial community composition.

The most interesting OTUs in the ordinations are the ones that land on the ends of the NMDS1 axis (Fig. 6). Eight OTUs were located on the same end as abiotic factors Cu and pH, and they thus seem to flourish at or withstand higher pH and Cu levels than other bacteria in the materials (Fig. 6). The eight OTUs belonged to phyla Chloroflexi (2/8), Acidobacteria (2/8), Bacteroidetes (2/8), Proteobacteria (1/8), and one was an unidentified OTU. Acidobacteria were further identified to order Gp6. Proteobacteria was a member of the class Gammaproteobacteria. Unfortunately, none of the eight OTUs could be identified to genus level.

The OTUs located 0.7 or further along the NMDS1 axis preferred lower levels of pH and Cu. Moss had the lowest level of each of these factors, and it is located at the furthest end of the NMDS1 axis and surrounded by 11 OTUs belonging to phyla Proteobacteria (4), Bacteroidetes (3), and Acidobacteria (4). Proteobacteria belonged to classes Beta- and Gammaproteobacteria represented by genera *Burkholderia*, *Rhodanobacter*, *Dyella,* and an unidentified genus within Gammaproteobacteria. Bacteroidetes belonged to genus *Mucilaginibacter* and family

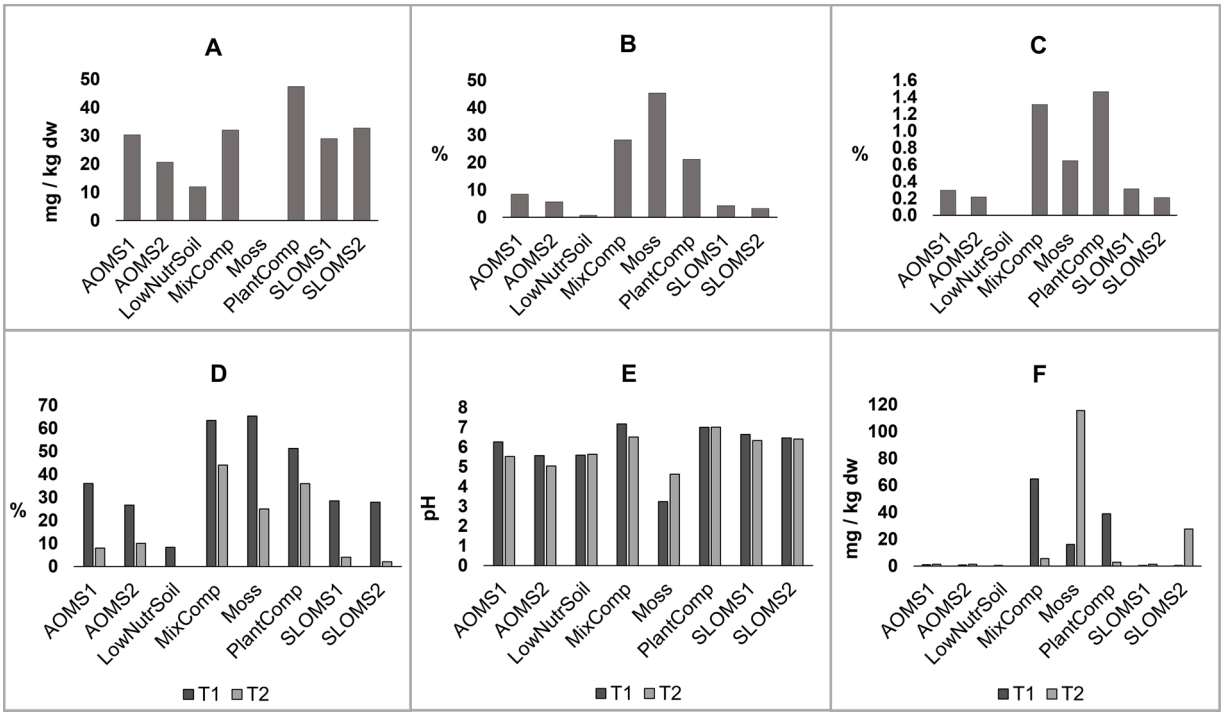

Fig. 5. Environmental factors that affected microbial communities at OTU level. Cu (A) levels (mg/kg dw) measured before storage varied between soil materials. Total C (B) and N (C) percentages did not change during storage, but they varied between soil materials as seen in the after storage measurements here. Water content percentage (D), pH (E), and NH_4+ (F) levels (mg/kg dw) were also visualized before (T1) and after (T2) storage. The figures were made using Excel (Microsoft Corporation, 2016).

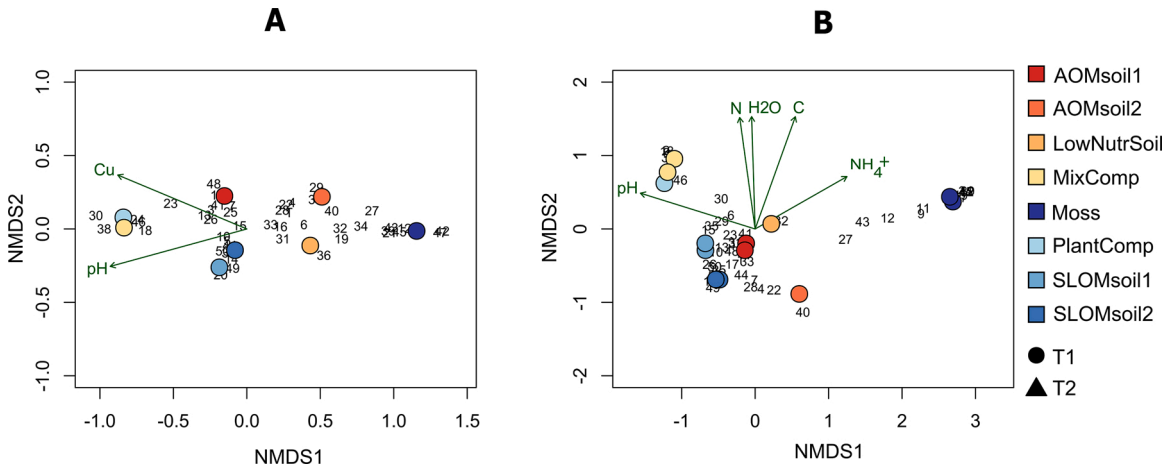

Fig. 6. OTU level NMDS ordinations for timepoints: a = T1 (stress: 0.015), b = T2 (stress: 0.024). Soils are colored as in the legend, numbers indicate OTUs. Soil chemical properties are shown as arrows pointing to the direction where their values increase (declining in the opposite direction). The length of the arrow shows the importance of the variable. For T2 samples, elements were not measured. Only significant variables are shown.

Chitinophagaceae. Acidobacteria were mostly classes GP1 and GP2; one was further identified as order Granulicella.

After the three-year storage, most of the 50 most abundant OTUs were the same as at the beginning of the storage in composted soils and moss samples; compare ordinations T1 and T2. Two of the abundant OTUs from genera *Sulfurimonas* (relative abundance T1 = 1.25 %) and *Paraperlucidibaca* (relative abundance T1 = 0.81 %) (Proteobacteria) were no longer found after storage.

4. Discussion

We hypothesized that storage would cause shifts in bacterial community composition and decrease bacterial diversity and richness due to competition over remaining resources. Concurrently, the relative abundance of genera potentially containing opportunistic pathogens would be reduced. Our results supported these hypotheses. First, shifts in bacterial communities were observed at all taxonomic levels. Shifts detected in our landscaping materials are in accordance with the classification by Fierer et al. (2007) who identified taxa as having characteristics of the r- and K-selection strategies; the r-selection strategy refers to fast-growing bacterial species that live in unstable environments, while K-selection refers to slow-growing bacterial species that live in more stable environments. In our study, the shifts in bacterial community composition were observed particularly in those landscaping materials that had the most extreme concentrations of resources, supporting the idea of labile carbon levels decreasing and leading to the prevalence of K-selection bacteria. In this context, our results are in accord with conclusions of Fierer et al. (2007). Acidobacteria increased when carbon sources became rarer during the long storage, while Betaproteobacteria and Bacteroidetes decreased simultaneously with the depletion of labile carbon sources (Fierer et al., 2007). These results support the view that storage under constant environmental conditions results in depletion of quickly utilizable resources. As a decrease was observed in Chao1 richness and Sobs, it is obvious that species richness declined during storage, and a core reason for this was the disappearance of rare OTUs during the storage. Our assumption is that rare OTUs flourished in highly specialized niches. These niches may have disappeared during storage when the inflow of nutrients, water, labile carbon, and rare elements was minimal and temperature did not fluctuate, unlike in the field and during composting. The abundant OTUs that were not detected after storage belonged to Proteobacteria, especially the OTU from the genus *Sulfurimonas* whose relative abundance before storage was 1.25 %. This OTU belonged to Epsilonproteobacteria, which had decreased by 100 % during storage. *Sulfurimonas* are chemoautotrophs and they are found in versatile habitats (Han and Perner, 2015). As landscaping materials had vastly different physicochemical characteristics at the beginning of our study, storage plausibly causes homogeneous shifts in the abiotic living environment of bacteria over a wide range of landscaping materials.

The fact that total C and N levels were constant throughout the storage period indicates that there was no significant atmospheric nitrogen fixation, denitrification, or massive production of volatile organics. Since total N and C did not change, these elements may have converted to another form. Although the amount of NH_4+ did not change between timepoints, it correlated with the bacterial community composition after storage. The elevated NH_4+ level in moss and the prominent difference in bacterial community composition in moss after storage may imply ammonification of the degrading plant matter and heterotrophic microscopic organisms, including multicellular Eukaryotes. Acidobacteria subdivisions abundant in the samples have differing preferences, and they are sensitive to changes in nutrient and pH conditions (Navarrete et al., 2015). Thus, it makes sense that OTUs belonging to these orders seem to be affected by pH and nutrient changes and pulled to different sides of the ordinations with the fitted environmental factors (Fig. 5).

The three-year storage resulted in interesting changes in the context of human and animal health. The fact that genomes associated with butanoate metabolism increased in abundance in landscaping materials may be beneficial. Gut butanoate-forming bacteria support immune modulation, and they are associated positively with mental health (Geirnaert et al., 2017; Valles-Colomer et al., 2019). However, it is still unclear whether external exposure to butanoate-producing bacteria within landscaping materials is beneficial to health. The relative abundance and richness of Gammaproteobacteria and the richness of Proteobacteria decreased during storage, which may indicate poorer health benefit. Particularly, the generic diversity of skin Gammaproteobacteria has been associated with immune modulation (Hanski et al., 2012). If landscaping materials are used to modify skin gammaproteobacterial community (Nurminen et al., 2018; Hui et al., 2019a; Puhakka et al., 2019), it may become necessary to have a short shelf-life in consumer stores or preferably build novel materials that are able to maintain gammaproteobacterial diversity over years.

As the relative abundance of genera potentially containing opportunistic pathogens mostly declined or remained constant, our study suggests that landscaping materials are safe to use after a relatively long storage, especially when stored at low temperatures to inhibit the growth of certain pathogens (Katz and Hammel, 1987). In this context, *Pseudonocardia* was an exception that was mainly observed after the

three-year storage period. Since there are only two reports of Pseudonocardia infecting humans (Taylor et al., 2001; Navarro-Martínez et al., 2017), it seems that this genus seldom targets humans, suggesting that our long-term storage was safe concerning infectious diseases. *Pseudonocardia* belongs to the phylum Actinobacteria, which increased in abundance during storage. Actinobacteria are known for their ability to degrade complex compounds, and their potential in bioremediation is recognized (Alvarez et al., 2017). Possibly, the increased signaling in functional orthologs indicating xenobiotic degradation (Table 2) is connected to the increase in Actinobacteria. In addition to *Pseudonocardia*, *Mycobacterium* species belonging to the same phylum can degrade several xenobiotics and other less labile carbon sources (Vorbeck et al., 1998; Moody et al., 2001; Pagnout et al., 2007), and in this context the observed elevation in this genus may indicate saprotrophic activities. The OTUs classified as *Mycobacterium* seemed to match with known species and unidentified strains according to the BLAST search. The matches raised no concern of pathogenicity since the OTUs even distantly matching with pathogenic species were of very low abundance and also matched with non-pathogenic species. Saprotrophic *Mycobacterium* species dwelling in soils are harmless, but it has been proposed that they are able to stimulate human immune system regulation (Rook et al., 2004). Our study thus hints that deprivation of labile carbon and nutrients could enrich mycobacteria beneficial to immunomodulation. If the role of mycobacteria in inhibition of allergic symptoms was confirmed in intervention trials, our results about opposite shifts in gammaproteobacterial and mycobacterial relative abundance during storage would need to be revisited.

The increased signals in functional orthologs indicating biosynthesis of antibiotics would support the hypothesis that the change in bacterial communities is at least partially due to competition. As organic landscaping materials typically contain labile carbon that boosts plant growth and as virtually all soils and moss contain bacteria eager to quickly consume freely available resources, it is understandable that labile carbon was consumed soon after the beginning of the storage period. Since most of the labile carbon could be consumed or altered to more complex substrates, the landscaping materials deteriorated to plant growth media during the storage. This hypothesis is further supported by the increased abundance of several functional orthologs attached to degradation of complex molecules such as xenobiotics. Several of the xenobiotics, such as atrazine and polycyclic aromatic hydrocarbons, are toxic to many micro-organisms, i.e. there may be a trade-off to use them as an energy or carbon source, and carbon and nitrogen in the xenobiotics are not easily unbound (Sinkkonen et al., 2013a; Mikkonen et al., 2018). Despite the fact that we did not perform a PCR analysis of each gene linked to functions in KEGG separately, estimating functions of a bacterial community from its genomic data allows us to make assumptions of microbial interactions during the degradation of organic landscaping materials (Kanehisa and Goto, 2000; Vari et al., 2021). In our study, these assumptions are in line with the taxonomic changes observed.

5. Conclusions

We conclude that a long storage period converts resources to a more complex form, possibly making them harder to utilize for plant seedlings. Importantly, a long storage period alters microbial community composition, and consequently, the potential health outcomes associated with active contacts with landscaping materials. Therefore, using fresh landscaping materials is advisable in urban greening, providing that the purpose is to maximize plant growth and the abundance of health-associated bacterial taxa. Since the storage conditions elevated butyrate formation and the relative abundance of mycobacteria, which could be beneficial, further studies regarding these taxa in relation to human health may promote the planning of immunomodulatory urban greening. As the absence of readily available resources enriched functional orthologs associated with xenobiotic degradation, our results pave the way for novel practices to treat landscaping materials in polluted environments. Regarding the growth of opportunistic bacterial strains, landscaping materials seem safe to use after long-term storage. Further research is needed to elucidate whether these results are applicable to different soil volumes and storage times, storage at higher temperatures, and landscaping materials originating at lower latitudes.

Availability of data and material

Sequence data are available in Sequence Read Archive.

Declaration of Competing Interest

The authors declare there are no competing interests.

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Appendix A. Organic matter percentage (OM%) and elements (mg / kg dw) in landscaping materials measured before storage in 2015

Landscaping material	OM %	Al	Co	Cr	Cu	Mn	Ni	Fe	Zn	P	Pb
SLOMsoil2	12	16644	7.3	36.5	32.8	282.6	17.4	24643.2	106.7	1570.5	5.5
SLOMsoil1	10	6587.8	3.7	15	29	162.7	9.4	12802.7	0	1887.4	6.1
PlantComp	33	10740.4	4	22.9	47.4	469.4	12.2	10001.9	240.9	2669.1	23.3
Moss	99	286.1	0	0	0	229.8	0	630.7	0	333.7	5.1
AOMsoil2	13	6879.7	4.3	12.6	20.6	137.6	0	11631.4	0	621.5	3.8
AOMsoil1	22	7079.7	4.3	12.2	30.3	152.8	0	11028.2	0	589.3	0
MixComp	54	10309.8	0	35.3	32	429.8	17.8	2700.9	168.6	4912.5	4.1
LowNutrSoil	2	3656	0	0	12	66.2	0	5445	0	247.5	0

Appendix B

Fig. B1

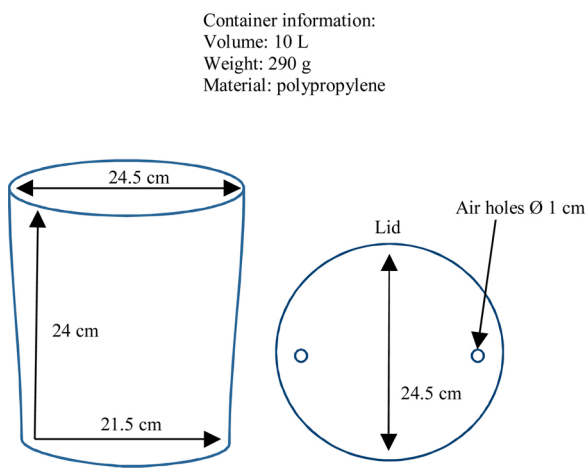

Fig. B1. Container information and dimensions.

Appendix C. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.ufug.2021.127065.

References

Alvarez, A., Saez, J.M., Davila Costa, J.S., Colin, V.L., Fuentes, M.S., Cuozzo, S.A., Benimeli, C.S., Polti, M.A., Amoroso, M.J., 2017. Actinobacteria: current research and perspectives for bioremediation of pesticides and heavy metals. Chemosphere 166, 41–62. https://doi.org/10.1016/j.chemosphere.2016.09.070.

Caporaso, J.G., Lauber, C.L., Walters, W.A., Berg-Lyons, D., Huntley, J., Fierer, N., Owens, S.M., Betley, J., Fraser, L., Bauer, M., Gormley, N., Gilbert, J.A., Smith, G., Knight, R., 2012. Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. ISME J. 6, 1621–1624. https://doi.org/10.1038/ismej.2012.8.

Cole, J.R., Wang, Q., Cardenas, E., Fish, J., Chai, B., Farris, R.J., McGarrell, D.M., Marsh, T., Garrity, G.M., Kulam-Syed-Mohideen, A.S., McGarrell, D.M., Marsh, T., Garrity, G.M., Tiedje, J.M., 2009. The Ribosomal Database Project: improved alignments and new tools for rRNA analysis. Nucleic Acids Res. 37, 141–145. https://doi.org/10.1093/nar/gkn879.

Cutler, N.A., Chaput, D.L., van der Gast, C.J., 2014. Long-term changes in soil microbial communities during primary succession. Soil Biol. Biochem. 69, 359–370. https://doi.org/10.1016/j.soilbio.2013.11.022.

De Groote, M.A., Pace, N.R., Fulton, K., Falkinham, J.O., 2006. Relationships between Mycobacterium isolates from patients with pulmonary mycobacterial infection and potting soils. Appl. Environ. Microbiol. 72, 7602–7606. https://doi.org/10.1128/AEM.00930-06.

DeSantis, T.Z., Hugenholtz, P., Larsen, N., Rojas, M., Brodie, E.L., Keller, K., Huber, T., Dalevi, D., Hu, P., Andersen, G.L., 2006. Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB. Appl. Environ. Microbiol. 72, 5069–5072. https://doi.org/10.1128/AEM.03006-05.

Edgar, R.C., Haas, B.J., Clemente, J.C., Quince, C., Knight, R., 2011. UCHIME improves sensitivity and speed of chimera detection. Bioinformatics 27, 2194–2200. https://doi.org/10.1093/bioinformatics/btr381.

Fierer, N., Bradford, Ma., Jackson, R.B., 2007. Toward an ecological classification of soil Bacteria. Ecology 88, 1354–1364. https://doi.org/10.1890/05-1839.

Flandroy, L., Poutahidis, T., Berg, G., Clarke, G., Dao, M.C., Decaestecker, E., Furman, E., Haahtela, T., Massart, S., Plovier, H., Sanz, Y., Rook, G., 2018. The impact of human activities and lifestyles on the interlinked microbiota and health of humans and of ecosystems. Sci. Total Environ. 627, 1018–1038. https://doi.org/10.1016/j.scitotenv.2018.01.288.

Geirnaert, A., Calatayud, M., Grootaert, C., Laukens, D., Devriese, S., Smagghe, G., De Vos, M., Boon, N., Van De Wiele, T., 2017. Butyrate-producing bacteria supplemented in vitro to Crohn's disease patient microbiota increased butyrate production and enhanced intestinal epithelial barrier integrity. Sci. Rep. 7, 1–14. https://doi.org/10.1038/s41598-017-11734-8.

Grönroos, M., Parajuli, A., Laitinen, O.H., Roslund, M.I., Vari, H.K., Hyöty, H., Puhakka, R., Sinkkonen, A., 2019. Short-term direct contact with soil and plant materials leads to an immediate increase in diversity of skin microbiota. Microbiologyopen. https://doi.org/10.1002/mbo3.645.

Han, Y., Perner, M., 2015. The globally widespread genus Sulfurimonas: versatile energy metabolisms and adaptations to redox clines. Front. Microbiol. 6, 1–17. https://doi.org/10.3389/fmicb.2015.00989.

Hanski, I., von Hertzen, L., Fyhrquist, N., Koskinen, K., Torppa, K., Laatikainen, T., Karisola, P., Auvinen, P., Paulin, L., Mäkelä, M.J., Vartiainen, E., Kosunen, T.U., Alenius, H., Haahtela, T., Makela, M.J., Vartiainen, E., Kosunen, T.U., Alenius, H., Haahtela, T., 2012. Environmental biodiversity, human microbiota, and allergy are interrelated. Proc. Natl. Acad. Sci. U. S. A. 109, 8334–8339. https://doi.org/10.1073/pnas.1205624109.

Herzog, C., Hartmann, M., Frey, B., Stierli, B., Rumpel, C., Buchmann, N., Brunner, I., 2019. Microbial succession on decomposing root litter in a drought-prone Scots pine forest. ISME J. 13, 2346–2362. https://doi.org/10.1038/s41396-019-0436-6.

Hui, N., Grönroos, M., Roslund, M.I., Parajuli, A., Vari, H.K., Soininen, L., Laitinen, O.H., Sinkkonen, A., 2019a. Diverse environmental microbiota as a tool to augment biodiversity in urban landscaping materials. Front. Microbiol. 10, 1–10. https://doi.org/10.3389/fmicb.2019.00536.

Hui, N., Parajuli, A., Puhakka, R., Grönroos, M., Roslund, M.I., Vari, H.K., Selonen, V.A.O., Yan, G., Siter, N., Nurminen, N., Oikarinen, S., Laitinen, O.H., Rajaniemi, J., Hyöty, H., Sinkkonen, A., 2019b. Temporal variation in indoor transfer of dirt-associated environmental bacteria in agricultural and urban areas. Environ. Int. 132, 105069 https://doi.org/10.1016/j.envint.2019.105069.

Huse, S.M., Welch, D.M., Morrison, H.G., Sogin, M.L., 2010. Ironing out the wrinkles in the rare biosphere through improved OTU clustering. Environ. Microbiol. 12, 1889–1898. https://doi.org/10.1111/j.1462-2920.2010.02193.x.

Kanehisa, M., Goto, S., 2000. KEGG: Kyoto encyclopedia of genes and genomes. Nucleic Acids Res. 28, 27–30.

Katz, S.M., Hammel, J.M., 1987. The effect of drying, heat, and pH on the survival of Legionella pneumophila. Ann. Clin. Lab. Sci. 17, 150–156.

Kirjavainen, P.V., Karvonen, A.M., Adams, R.I., Täubel, M., Roponen, M., Tuoresmäki, P., Loss, G., Jayaprakash, B., Depner, M., Ege, M.J., Renz, H., Pfefferle, P.I., Schaub, B., Lauener, R., Hyvärinen, A., Knight, R., Heederik, D.J.J., von Mutius, E., Pekkanen, J., 2019. Farm-like indoor microbiota in non-farm homes protects children from asthma development. Nat. Med. 25, 1089–1095. https://doi.org/10.1038/s41591-019-0469-4.

Kozich, J., Westcott, S., Baxter, N., Highlander, S., Schloss, P., 2013. Development of a dual-index sequencing strategy and curation pipeline for analyzing amplicon sequence data on the MiSeq Illumina sequencing platform. Appl. Environ. Microbiol. 79, 5112–5120.

Krzywinski, M., Schein, J., Birol, I., Connors, J., Gascoyne, R., Horsman, D., Jones, S.J., Marra, M.A., 2009. Circos: an information aesthetic for comparative genomics. Genome Res. 19, 1639–1645. https://doi.org/10.1101/gr.092759.109.

Langille, M.G.I., Zaneveld, J., Caporaso, J.G., McDonald, D., Knights, D., Reyes, J.A., Clemente, J.C., Burkepile, D.E., Vega Thurber, R.L., Knight, R., Beiko, R.G., Huttenhower, C., 2013. Predictive functional profiling of microbial communities using 16S rRNA marker gene sequences. Nat. Biotechnol. 31, 814–821. https://doi.org/10.1038/nbt.2676.

Lauber, C.L., Zhou, N., Gordon, J.I., Knight, R., Fierer, N., 2010. Effect of storage conditions on the assessment of bacterial community structure in soil and human-associated samples. FEMS Microbiol. Lett. 307, 80–86. https://doi.org/10.1111/j.1574-6968.2010.01965.x.

McMurdie, P.J., Holmes, S., 2013. Phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. PLoS One 8, e61217. https://doi.org/10.1371/journal.pone.0061217.g004.

Microsoft Corporation, 2016. Excel.

Mikkonen, A., Yläranta, K., Tiirola, M., Dutra, L.A.L., Salmi, P., Romantschuk, M., Copley, S., Ikäheimo, J., Sinkkonen, A., 2018. Successful aerobic bioremediation of groundwater contaminated with higher chlorinated phenols by indigenous degrader bacteria. Water Res. 138, 118–128. https://doi.org/10.1016/j.watres.2018.03.033.

Moody, J.D., Freeman, J.P., Doerge, D.R., Cerniglia, C.E., 2001. Degradation of Phenanthrene and anthracene by cell suspensions of Mycobacterium sp. Strain PYR-1. Appl. Environ. Microbiol. 67, 1476–1483. https://doi.org/10.1128/AEM.67.4.1476-1483.2001.

Navarrete, A.A., Venturini, A.M., Meyer, K.M., Klein, A.M., Tiedje, J.M., Brendan, B.J., Nüsslein, K., Tsai, S.M., Rodrigues, J.L.M., 2015. Differential response of Acidobacteria subgroups to forest-to-pasture conversion and their biogeographic patterns in the western Brazilian Amazon. Front. Microbiol. 6, 1–10. https://doi.org/10.3389/fmicb.2015.01443.

Navarro-Martínez, A., Corominas, N., Sainz de Baranda, C., Escudero-Jiménez, Á., Galán-Ros, J., Sáez- Nieto, J.A., Solera, J., 2017. Pseudonocardia carboxydivorans in human cerebrospinal fluid: a case report in a patient with traumatic brain injury. BMC Infect. Dis. 17, 1–4. https://doi.org/10.1186/s12879-017-2538-y.

Nurminen, N., Lin, J., Grönroos, M., Puhakka, R., Kramna, L., Vari, H.K., Viskari, H., Oikarinen, S., Roslund, M., Parajuli, A., Tyni, I., Cinek, O., Laitinen, O., Hyöty, Heikki, Hyöty, Hÿ, Sinkkonen, A., 2018. Nature-derived microbiota exposure as a novel immunomodulatory approach. Future Microbiol. 13, 737–744. https://doi.org/10.2217/fmb-2017-0286.

Oksanen, J., Blanchet, F.G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., Minchin, P.R., O'Hara, R.B., Simpson, G.L., Solymos, P., Stevens, M.H.H., Szoecs, E., Wagner, H., 2019. vegan: Community Ecology Package..

Orsini, F., Kahane, R., Nono-Womdim, R., Gianquinto, G., 2013. Urban agriculture in the developing world : a review. Agron. Sustain. Dev. 33, 695–720. https://doi.org/10.1007/s13593-013-0143-z.

Pagnout, C., Frache, G., Poupin, P., Maunit, B., Muller, J.F., Férard, J.F., 2007. Isolation and characterization of a gene cluster involved in PAH degradation in Mycobacterium sp. Strain SNP11: expression in Mycobacterium smegmatis mc2155. Res. Microbiol. 158, 175–186. https://doi.org/10.1016/j.resmic.2006.11.002.

Parajuli, A., Grönroos, M., Kauppi, S., Pøociniczak, T., Roslund, M., Galitskaya, P., Laitinen, O.H., Hyöty, H., Ari, Jumpponen, Rauni, Strömmer, Martin, Romantschuk, Nan, Hui, Aki, Sinkkonen, 2017. The abundance of health-associated bacteria is altered in PAH polluted soils—implications for health in urban areas? PLoS One 1–18. https://doi.org/10.1371/journal.pone.0187852.t001.

Parajuli, A., Grönroos, M., Siter, N., Puhakka, R., Vari, H.K., Roslund, M.I., Jumpponen, A., Nurminen, N., Laitinen, O.H., Hyöty, H., Rajaniemi, J., Sinkkonen, A., 2018. Urbanization reduces transfer of diverse environmental microbiota indoors. Front. Microbiol. 9. https://doi.org/10.3389/fmicb.2018.00084.

Parajuli, A., Hui, N., Puhakka, R., Oikarinen, S., Grönroos, M., Selonen, V.A.O., Siter, N., Kramna, L., Roslund, M.I., Vari, H.K., Nurminen, N., Honkanen, H., Hintikka, J., Sarkkinen, H., Romantschuk, M., Kauppi, M., Valve, R., Cinek, O., Laitinen, O.H., Rajaniemi, J., Hyöty, H., Sinkkonen, A., 2020. Yard vegetation is associated with gut microbiota composition. Sci. Total Environ. 713. https://doi.org/10.1016/j.scitotenv.2020.136707.

Pruesse, E., Quast, C., Knittel, K., Fuchs, B.M., Ludwig, W., Peplies, J., Glöckner, F.O., 2007. SILVA: a comprehensive online resource for quality checked and aligned ribosomal RNA sequence data compatible with ARB. Nucleic Acids Res. 35, 7188–7196. https://doi.org/10.1093/nar/gkm864.

Puhakka, R., Rantala, O., Roslund, M., Rajaniemi, J., Laitinen, O., Sinkkonen, A., 2019. Greening of daycare yards with biodiverse materials affords well-being, play and environmental relationships. Int. J. Environ. Res. Public Health 16, 2948. https://doi.org/10.3390/ijerph16162948.

R Core Team, 2019. R: A Language and Environment for Statistical Computing..

Rook, G.A.W., 2009. Review series on helminths, immune modulation and the hygiene hypothesis: the broader implications of the hygiene hypothesis. Immunology 126, 3–11. https://doi.org/10.1111/j.1365-2567.2008.03007.x.

Rook, G.A.W., Adams, V., Hunt, J., Palmer, R., Martinelli, R., Brunet, L.R., 2004. Mycobacteria and other environmental organisms as immunomodulators for immunoregulatory disorders. Springer Semin. Immunopathol. 25, 237–255. https://doi.org/10.1007/s00281-003-0148-9.

Roslund, M.I., Grönroos, M., Rantalainen, A.-L.L., Jumpponen, A., Romantschuk, M., Parajuli, A., Hyöty, H., Laitinen, O., Sinkkonen, A., 2018. Half-lives of PAHs and temporal microbiota changes in commonly used urban landscaping materials. PeerJ 2018, 1–27. https://doi.org/10.7717/peerj.4508.

Roslund, M.I., Rantala, S., Oikarinen, S., Puhakka, R., Hui, N., Parajuli, A., Laitinen, O. H., Hyöty, H., Rantalainen, A.L., Sinkkonen, A., the ADELE team, 2019. Endocrine disruption and commensal bacteria alteration associated with gaseous and soil PAH contamination among daycare children. Environ. Int. 130, 104894 https://doi.org/10.1016/j.envint.2019.06.004.

Rubin, B.E.R., Gibbons, S.M., Kennedy, S., Hampton-Marcell, J., Owens, S., Gilbert, J.A., 2013. Investigating the impact of storage conditions on microbial community composition in soil samples. PLoS One 8, 1–9. https://doi.org/10.1371/journal.pone.0070460.

Schloss, P.D., Gevers, D., Westcott, S.L., 2011. Reducing the effects of PCR amplification and sequencing Artifacts on 16s rRNA-based studies. PLoS One 6. https://doi.org/10.1371/journal.pone.0027310.

SFS 3008, 1990. Determination of Dry Matter and Loss on Ignition for Water, Sludge and Sediment.

SFS-ISO 10390, 2005. Soil quality determination of pH..

Sinkkonen, A., Kauppi, S., Pukkila, V., Nan, H., Płociniczak, T., Kontro, M., Strömmer, R., Romantschuk, M., 2013a. Previous exposure advances the degradation of an anthropogenic s-triazine regardless of soil origin. J. Soils Sediments 13, 1430–1438. https://doi.org/10.1007/s11368-013-0742-y.

Sinkkonen, A., Kauppi, S., Simpanen, S., Rantalainen, A.-L., Strömmer, R., Romantschuk, M., 2013b. Layer of organic pine forest soil on top of chlorophenol-contaminated mineral soil enhances contaminant degradation. Environ. Sci. Pollut. Res. 20, 1737–1745. https://doi.org/10.1007/s11356-012-1047-1.

Taylor, L.H., Latham, S.M., Woolhouse, M.E.J., 2001. Risk factors for human disease emergence. Philos. Trans. R. Soc. B Biol. Sci. 356, 983–989. https://doi.org/10.1098/rstb.2001.0888.

Valles-Colomer, M., Falony, G., Darzi, Y., Tigchelaar, E.F., Wang, J., Tito, R.Y., Schiweck, C., Kurilshikov, A., Joossens, M., Wijmenga, C., Claes, S., Van Oudenhove, L., Zhernakova, A., Vieira-silva, S., Raes, J., 2019. Microbiota in quality of life and depression. Nat. Microbiol. https://doi.org/10.1038/s41564-018-0337-x.

Vari, H.K., Roslund, M.I., Oikarinen, S., Nurminen, N., Puhakka, R., Parajuli, A., Grönroos, M., Siter, N., Laitinen, O.H., Hyöty, H., Rajaniemi, J., Rantalainen, A.-L., Sinkkonen, A., 2021. Associations between land cover categories, gaseous PAH levels in ambient air and endocrine signaling predicted from gut bacterial metagenome of the elderly. Chemosphere. https://doi.org/10.1016/j.chemosphere.2020.128965.

Von Hertzen, L., Haahtela, T., 2006. Disconnection of man and the soil: reason for the asthma and atopy epidemic? J. Allergy Clin. Immunol. 117, 334–344. https://doi.org/10.1016/j.jaci.2005.11.013.

Von Hertzen, L., Hanski, I., Haahtela, T., 2011. Natural immunity. Biodiversity loss and inflammatory diseases are two global megatrends that might be related. EMBO Rep. 12, 1089–1093. https://doi.org/10.1038/embor.2011.195.

Vorbeck, C., Lenke, H., Fischer, P., Spain, J.C., Knackmuss, H.J., 1998. Initial reductive reactions in aerobic microbial metabolism of 2,4,6- trinitrotoluene. Appl. Environ. Microbiol. 64, 246–252.

Wang, Q., Garrity, G.M., Tiedje, J.M., Cole, J.R., Al, W.E.T., 2007. Naï Ve Bayesian Classifier for Rapid Assignment of rRNA Sequences into the New Bacterial Taxonomy, Vol 73, pp. 5261–5267. https://doi.org/10.1128/AEM.00062-07.

Zhang, Z., Schwartz, S., Wagner, L., Miller, W., 2000. A greedy algorithm for aligning DNA sequences. J. Comput. Biol. 7, 203–214. https://doi.org/10.1089/10665270050081478.